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### Research Article

## Comparative assessment optimization and characterization of bioactive constituents from *Amarantus* species: An indigenous lesser-known vegetables of India

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### Abstract

*Amaranthus Tricolor L.* (red amaranth) and *Amaranthus Viridis* (Green amaranth) are *Amaranthaceae* members, widely cultivated in Asia and consumed as a leafy vegetable in many parts of the world. This study deal with the nutritional and functional, and biochemical characterization of *A. Tricolor* and *A. viridis*. Single-factor experiments and Box Behnken Design (BBD) were used to optimize the extraction process for the phenolic compound from *A. Tricolor* and *A. viridis*. The BBD shows that 11.87mg/g phenolic extract of *A. Tricolor* produced at the optimal condition of solid to water ratio (1:15), temperature (30°C), and time (15 min). Similarly, *A. viridis* isolated 18.36mg/g of phenolics at optimal condition solid to liquid ratio (1:30), temperature (30°C), and time (60 min). The radical scavenging activity of *A. Tricolor* and *A. viridis* shows 63.52% and 19.27%, respectively, by the DPPH method. The bioactive compounds 3,4,5-Trihydroxystilbene and caffeic acid were found in *A. Tricolor*, and in *A. viridis* it showed caffeic acid, which was identified using LC-MS/MS.

**Keywords:** *Amaranthus Tricolor L.*, *Amaranthus Viridis*, polyphenols, BBD, antioxidant activity

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## Introduction

Small, frequently green or reddish flowers clustered in dense clusters, strongly coloured stems and leaves, and dry, indehiscent, one-seeded fruit define *Amaranth spp.* The genus *Amaranthus* contains around 60 species that are found throughout the world in temperate, subtropical, and tropical climates, with at least 17 species having edible leaves and three grain amaranths. *Amaranthus* is a widely grown plant with a high genetic and particular diversity that is used for food (as a pseudo cereal plant), feeding, and ornamentation. Despite the fact that some species are commonly considered weeds, amaranths are valued as green vegetables, grains, and ornamentals by people all across the world. *Amaranthus* species are widely distributed throughout the world's temperate and tropical regions even before man converted some of them into cosmopolitan weeds and domesticated others (Das 2016). Schröter et al. (2018) provided information about presence of secondary metabolites in six different species of Amaranth, which strongly recommended to consider crop for food consumption which delivers the health benefits associated with it.

*Amaranthus Tricolor L.* (red amaranth) and *Amaranthus Viridis* (green amaranth) are leafy vegetables produced mainly in Africa and Asia, which belong to the family *Amaranthaceae*. *Amaranthus tricolor L.* (*A. tricolor*) leaves consist of health beneficiaries bioactive compounds such as antioxidants, polyphenols, and betacyanin. These could be a potential source for anti-inflammatory and antioxidant (Khandaker et al. 2008). These are also known for protein sources with a balance of essential amino acids, minerals (iron and calcium), and vitamin C (Islam et al. 2003).

*Amaranthus Viridis* (*A. viridis*) reported bioactive properties such as antioxidant activity, phenolic acid, and improved nutritional quality due to the drought stress (Sarkar and Oba 2018). *A. viridis* is a good source of antioxidants, phenolic acids, and vitamin C (Datta et al. 2019). The extract of *A. viridis* has been utilized in the bread fortification to enrich with a polyphenolic group with increasing antioxidant properties (Alashi et al. 2018).

The various pharmaceutical, food, and beverage industries demanded natural antioxidant from plant sources, as demand increases as ingredient/ dietary

supplements or functional food to avoid the effect of synthetic antioxidants on human health (Ilaiyaraja et al. 2015; Arnáiz et al. 2016). Polyphenols playing key role in the health benefits which contribute as antihypertensive, anti-obesity, anti-diabetic, anti-hyperlipidemic and anti-inflammatory effects (Cherniac 2011). Polyphenols are thought to be good for your health, therefore they could be used in new ways to prevent diabetes and obesity issues (Mihaylova et al. 2018).

Solvent extraction is an efficient method widely used to isolate phenolic compounds from the fruits and vegetable sources with a maximum yield of target compounds with the highest quality and antioxidant activity (Spigno et al. 2007). The increased extraction efficiency for bioactive compounds is influenced by using a combination of solvents. Advanced techniques like pressurized liquid extraction are used nowadays to extract bioactive compounds from plant sources which are observed to more efficient (Li et al. 2002; Petkova et al. 2020; Trifonova et al., 2021). Water and ethanol and a combination of the two were the most popular solvents for extracting bioactive chemicals for food applications (Mihaylova and Lante, 2019). Lante et al. (2018) utilized water as solvent for extraction of soy isoflavones. The various extraction parameters like solvent, temperature, time, particle size, and solid-to-liquid ratio impact extraction (Dent et al. 2013).

A technological approach to extracting polyphenols from *A. Tricolor* and *A. viridis* is lacking. The primary goal of this study was to determine the best processing parameter for extracting phenolic content from *A. Tricolor* and *A. viridis* and compare the results using the Box-Behnken design.

## Materials and Methods

### Chemicals

Methanol, ethanol, acetone, gallic acid, HCl, L-ascorbic acid, petroleum ether (PET), folin-ciocalteau reagent, quercetin, sodium hydroxide, boric acids were purchased from SRL Pvt Ltd (Mumbai, Maharashtra, India). 1-Diphenyl-2-Picrylhydrazyl (DPPH) purchased from Sigma-Aldrich, India. All chemicals used in the experiment were analytical grade.

### A. *tricolor* and A. *viridis* powder preparation

A. *tricolor* and A. *viridis* were collected from APMC market, Navi Mumbai, India (a local market) in one lot. The washed and clean leaves of vegetables were dried overnight at 60°C. These leaves ground and powder were stored in polyethylene bags at 4°C to carried out research uniformly (Sonawane and Arya 2015).

### Proximate analysis of A. *tricolor* and A. *viridis*

The AOAC (1995) methods estimate moisture, fat, protein, fat, and mineral from A. *tricolor* and A. *viridis*.

### Analysis of moisture content

The samples were analyzed for their moisture content by heating at about 105°C in a hot air oven for 5 h (AOAC Official method 931.04 – 31.1.02).

### Analysis of fat content

The crude lipids were estimated by the Soxhlet extraction method (using instant Soxhlet apparatus - Socs Plus, Pelican equipment, Chennai, India). The fat was extracted using petroleum ether (b.p. 60-80°C) as solvent (AOAC Official method 920.39 –4.5.01).

### Analysis of ash

The samples were analyzed for their mineral content by heating it in a muffle furnace at about 570°C for 5 h (AOAC Official method 972.15 – 31.1.04). The charred mass left behind stands for the ash content of samples.

### Analysis of protein

The protein content of samples was analyzed by determining the nitrogen content by Kjeldahl method and then using a conversion factor of 6.25 (AOAC Official method 970.22 – 31.1.08).

### Carbohydrate content

Finally, the carbohydrate content of samples was calculated on a dry weight basis by a difference as follows.

$$\text{Percentage carbohydrate content} = 100 - (\% \text{ Fat} + \% \text{ Ash} + \% \text{ Protein})$$

### Experimental design for extraction of polyphenols from A. *tricolor* and A. *viridis*

The extraction of polyphenols from A. *tricolor* and A. *viridis* to obtain from preliminary data (not shown) with a suitable range for temperature (30 to 50°C), time range for A. *tricolor* (15 to 45 min), and for A. *viridis* it ranges from 60 to 120min, solid to solvent ratio A. *tricolor* (1:10 to 1:30) and for A. *viridis* it ranges from for (1:30 to 1:50). In preliminary data, water was suitable solvent to extract maximum amount of polyphenols. A three-level-three-factor BBD (Box-Behnken design, Design-Expert1 6 software, Stat-Ease Inc., Minneapolis, USA) was employed to enhance extraction parameters for polyphenols from A. *tricolor* and A. *viridis*. These designed used various combinations between temperature (A, °C), time (B, min), and the ratio of solid to liquid (C, w/v) to enhance the extraction of polyphenols from A. *tricolor* and A. *viridis*. These provide 17 combinations for the experiment using the above extraction parameters to keep total phenolic content, as shown in Table 1.

The experimental data obtained were fitted by the following regression equation:

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^3 \quad (1)$$

Whereas Y is the total phenolic content (predicted response),  $\beta_0, \beta_1, \beta_2, \dots, \beta_{33}$ , where the regression coefficient, A-temperature, B-time, C-solid to liquid.

**Table 1.** Experimental design for *A. tricolor* and *A. viridis*

Run	<i>A. tricolor</i>				<i>A. viridis</i>			
	Temperature	Time	Solid to Liquid	TPC	Temperature	Time	Solid to Liquid	TPC
1	30.00	15.00	20.00	10.86± 0.23	30.00	60.00	40.00	17.35±0.44
2	50.00	15.00	20.00	9.94± 0.85	50.00	60.00	40.00	19.79±1.05
3	30.00	45.00	20.00	8.85± 0.83	30.00	120.00	40.00	16.99±0.14
4	50.00	45.00	20.00	12.65±1.06	50.00	120.00	40.00	20.09±1.91
5	30.00	30.00	10.00	8.34± 0.25	30.00	90.00	30.00	16.77±1.33
6	50.00	30.00	10.00	8.86± 0.68	50.00	90.00	30.00	19.01±0.98
7	30.00	30.00	30.00	7.55± 0.53	30.00	90.00	50.00	15.71±1.85
8	50.00	30.00	30.00	8.56± 0.83	50.00	90.00	50.00	17.01±1.56
9	40.00	15.00	10.00	9.15± 0.15	40.00	60.00	30.00	19.59±0.67
10	40.00	45.00	10.00	9.75± 0.43	40.00	120.00	30.00	19.8±0.33
11	40.00	15.00	30.00	9.56± 0.76	40.00	60.00	50.00	17.71±0.52
12	40.00	45.00	30.00	9.27± 0.48	40.00	120.00	50.00	17.34±1.15
13	40.00	30.00	20.00	11.49± 0.94	40.00	90.00	40.00	13.27±0.66
14	40.00	30.00	20.00	11.52± 0.43	40.00	90.00	40.00	13.46±0.91
15	40.00	30.00	20.00	11.1± 0.56	40.00	90.00	40.00	13.85±0.85
16	40.00	30.00	20.00	11.29± 0.74	40.00	90.00	40.00	13.03±1.01
17	40.00	30.00	20.00	11.19± 0.98	40.00	90.00	40.00	13.67±0.95

\*Data are expressed as mean ± standard deviation

#### Characterization of polyphenols from *A. tricolor* and *A. viridis*

##### Total phenolic content of *A. tricolor* and *A. viridis* extract

The phenolic content of extracts from *A. tricolor* and *A. viridis* was investigated using the Folin-ciocalteu method described by Singh et al. 2002; Sonawane and Arya, 2013; 2015. The results were reported in mg of GAE/g of a sample.

##### Total flavonoid estimation in *A. tricolor* and *A. viridis* extract

The estimation of total flavonoid content in the extract of *A. tricolor* and *A. viridis* was carried out using Parimala and Shoba (2013). The extract (0.5mL) mixed with methanol (4.5mL) with addition of 10% aluminium chloride (0.1mL).

Further, potassium acetate (0.1mL) was added to the mixture and kept in the dark for 30min incubation, and absorption was measured at 415nm. The results expressed as quercetin equivalent (mg/g).

##### Estimation of tannin content in *A. tricolor* and *A. viridis* extract

The estimation of tannin in *A. tricolor* and *A. viridis* was carried out using Mohan et al. (2016). Initially, 0.2g of *A. tricolor* and *A. viridis* was taken with 70% aqueous acetone (10 mL) for 30°C for two hours in an ice bath shaker to extract tannin. The supernatant was collected by using a centrifuge at 8000 RPM. The 0.2mL of extract+0.8ml of distilled water+0.5ml Folin-ciocalteu's reagent+2.5ml of sodium carbonate (20%) was incubated for 40 min, and absorbance measured at 725 nm.

### Antioxidant activity estimation in *A. tricolor* and *A. viridis* extract

Antioxidant capacity of *A. tricolor* and *A. viridis* extract were measured by using DPPH (radical scavenging activity) reported by [Sonawane and Arya \(2015\)](#) and [Sahreen et al. \(2012\)](#), and results are expressed as % radical scavenging activity by using the following formula:

$$\text{RSA (\%)} = \frac{A-B}{A} \times 100$$

where:

A- Initial absorption of DPPH

B-Final absorption of the mixture

### Polyphenol profiling by using LC-QToF-MS/MS analysis

LC-ESI-Q-TOF-MS analysis *A. tricolor* and *A. viridis* leave extract using an Agilent 6200 (CA, USA) series Liquid Chromatography system. A Luna(r) C18 column (5  $\mu\text{m}$ , 150x2 mm) (Phenomenex) using DI water and acetonitrile in the ratio 95:5 (v/v) while applying the gradient at 2-15 min (95:5, v/v), 16-24 min (5:95, v/v), 25-30 min (95:5, v/v) at the flow rate of 0.2 ml/min for 30 min. The injection volume was set at three  $\mu\text{L}$  with a total run time of 30 min. An Agilent G6550A ultra high definition Accurate-Mass Quadrupole Time of Flight Mass Spectrophotometer using Agilent Mass Hunter Software version B.05.01 (B5125) was employed. The Agilent personal Compound Database Library (PCDL) version B.05.01 build 92 was used to create the custom database ([Kadam et al., 2018](#)).

### Statistical analysis

All experiments were performed in triplicate, and the results were expressed as mean  $\pm$  standard deviation. The SPSS (Statistical Package for Social Sciences) for Windows version (16.0) was used to analyze the data (SPSS Inc., Chicago, IL). Statistical significance was considered at  $p < 0.05$ .

## Results and Discussion

### Proximate analysis

*A. tricolor* contained 1.77% ash, 0.72% fat, 3% protein, and 94.51% carbohydrates by difference on a dry basis, which was comparatively higher to *A. viridis* (1.55% ash, 0.42% fat, 1.99% protein, and 96.04% carbohydrates). These findings are in the range reported by [Schonfeldt and Pretorius \(2011\)](#), [Sharma et al. \(2012\)](#) and [Lintas \(1992\)](#).

### Response surface methodology

The effect of extraction parameters such as temperature (A), time (B), and solid-to-liquid ratio (C) on phenolic material extraction from *A. tricolor* and *A. viridis* leaves was investigated using a BBD design. The one factor that helps in designing an experiment based on total phenolic content as a response. Table 1 shows the experimental outcomes obtained by design. The various combinations of extraction parameters led to the extraction of phenolic contents varying from 7.55 to 12.65 mg/g for *A. tricolor* and 13.03 and 20.09 mg/g for *A. viridis*.

### Model fitting

Furthermore, in our experiment, efforts were made to investigate interaction of temperature, time, and solid to liquid ratio on the total phenolic content using BBD. The ANOVA of the polyphenolic extract is shown in Table 2 which shows that model is fitted for the extraction parameters. The best explanatory model provided in Eq. (1) is given in Eq. (2) and (3) for *A. tricolor* and *A. viridis*, respectively, which corresponds to total phenolic content.

$$\text{TPC} = 11.32 + 0.55 A + 0.13 B - 0.15 C + 1.18 AB + 0.12 AC - 0.22 BC - 0.92A^2 + 0.18 B^2 - 2.07 C^2 \quad (2)$$

$$\text{TPC} = 13.46 + 1.13 A - 0.028 B - 0.93 C + 0.17 AB - 0.24 AC - 0.15BC + 1.8 A^2 + 3.29 B^2 + 1.86 C^2 \quad (3)$$

The lack of fit test for *A. tricolor* was an insignificant p-value of 0.1184) which indicates data in the experiment fitted well and acceptable to describe observed data. The results are presented in Table 3. The  $R^2 = 0.9838$  suggested that the fitted model can explain 98.38% of the variation in the data.



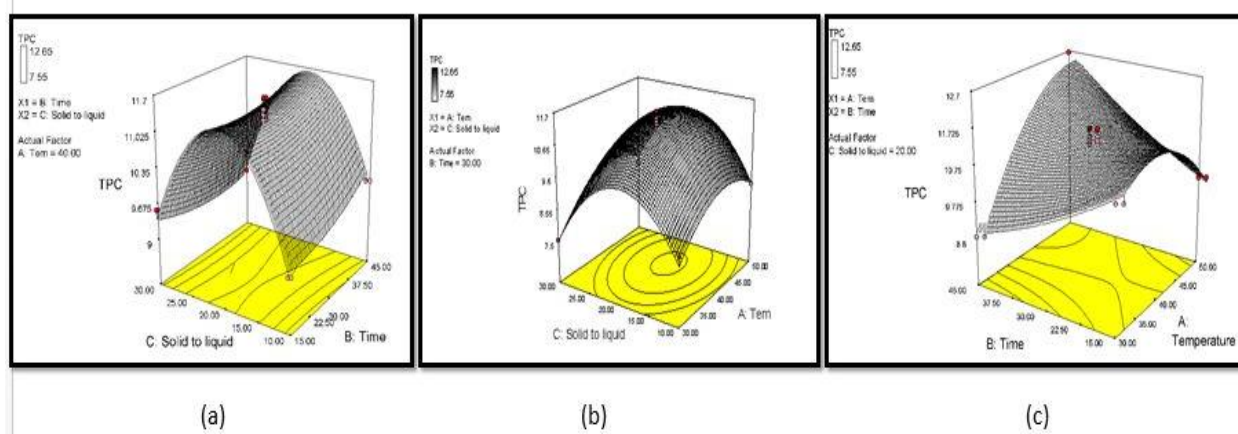
**Table 2.** ANOVA and Validation for *A. tricolor* and *A. viridis*

ANOVA		
Source	<i>A. tricolor</i>	<i>A. viridis</i>
Model (Prob > F)	< 0.0001	< 0.0001
Significant		
R-Squared	0.9838	0.9888
Adj R-Squared	0.9629	0.9744
Pred R-Squared	0.8022	0.8810
C.V. %	2.71	2.39
Lack of Fit	Insignificant	Insignificant
Validation of Model		
Parameters	<i>A. Tricolor</i>	<i>A.viridis</i>
Temperature, °C	30	30
Time, min	15	60
Solid to liquid, ml	15	30
Predicted TPC, mg/g	10.58	20.02
Experimental TPC, mg/g	11.87±0.21	18.36± 0.19

**Table 3.** Phytochemical analysis of *A. tricolor* and *A. viridis*

<i>Amaranthus species</i>	TPC, mg/g	DPPH, % RSA	Flavonoids, mg/g	Tannin, mg/g
<i>A.tricolor</i>	11.87±0.21	63.52±0.67	7.23±0.09	0.57±0.35
<i>A.viridis</i>	18.36±0.19	19.87±0.60	14.11±0.03	0.97±0.64

\*Data are expressed as mean ± standard deviation



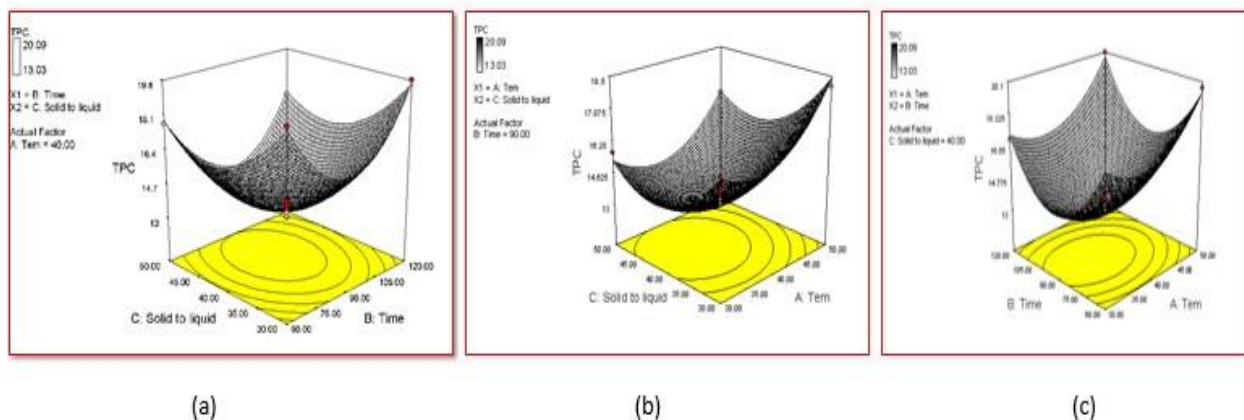
**Figure 1.** Response surface plot: effect of a solid to liquid and time b solid to liquid and temperature c time and temperature on total phenolic content of leaves extract from *A. tricolor*

Also, a high correlation was observed between Adj  $R^2$  (0.9629) and Pred  $R^2$  (0.8022). If the value of  $R^2$  is more or equal to 0.80 shows that the model is suitable for directing design space (Guan and Yao 2008). This model had a CV (coefficient of variation) of 2.71 %, suggesting an excellent precision and high reliability of the experiment achieved. Similarly, for *A. viridis*, the lack of fit test shows that the model was insignificant (p-value was 0.2239), indicating data in the analysis fitted well and acceptable to describe observed data (shown in Table 2). The  $R^2 = 0.9888$  suggested that the fitted model can explain 98.88% of the variation in the data. Also, a high correlation was observed between Adj  $R^2$  (0.9744) and Pred  $R^2$  (0.8810). This model had a CV (coefficient of variation) of 2.39%, suggesting an excellent precision and high reliability of the experiment achieved.

### Interpretation of the response surface model

The models' three-dimensional response surface and contour plots are shown by graphical representation in Figure 1 and Figure 2 which shows the interaction between each parameter to the corresponding response of the phenolic content of *A. tricolor* and *A. viridis*. Figure 1 (a) shows that the highest solid to

solvent ratio and extraction time of *A. tricolor* shows the lowest amount of phenolic content was extracted. Also, as solid to liquid ratio concentration increases, phenolic extraction increases up to a certain level and further decreases as the solid to liquid ratio increases. Figure 1 (b) shows the interactive effect of extraction temperature and solid to liquid ratio. The temperature above 40°C led to a decrease in the phenolic content as solid to liquid ratio increases above 1:20. But, in the case of Figure 1 (c), interactive plots show that higher extraction time and temperature lead to degradation of phenolic content in *A. tricolor*. A similar kind of response of phenolic extraction for the interaction of solid to solvent ratio, extraction time, and the temperature observed in *Solanum macrocarpon* (Famuwagun et al. 2017). In *A. viridis*, Figure 2 (a) shows that total phenolic concentration decreases as an increase in the solid to liquid ratio and time. In Figure 2 (b), interactive plots, solid to liquid ratio versus temperature shows that, as solid to liquid ratio increases, which decrease the phenolic content, but increase in the temperature leads to enhancement of the phenolic extraction. But, time and temperature interaction in Figure 2(c) shows that if it is increases which lead to decrease in the phenolic content.



**Figure 2.** Response surface plot: effect of a) solid to liquid and time b) solid to liquid and temperature c) time and temperature on total phenolic content of leaves extract from *A. viridis*

### Validation of experiments

Table 2 represents the validation of the experiment for the optimization of the extraction parameter for *A. tricolor* and *A. viridis*. In the case of *A. tricolor*, the extraction of phenolic content parameters shows a predicted value of 10.58 mg/g at the optimal

temperature 30°C, time 15 min, and solid to liquid 15ml as experimentally found as 11.87 mg/g, which confirms validation. Similarly, 18.36 mg/g was produced at the experimental condition of temperature 30°C, time 60 min, and solid to liquid 30ml, where it was predicted as 20.02 (mg/g).

### Characterization of a polyphenolic extract of *A. tricolor* and *A. viridis*

The polyphenolic composition of an extract of *A. tricolor* and *A. viridis* is shown in Table 3. The extract of *A. tricolor* and *A. viridis* contained 11.87 mg/g and 18.36 mg/g of total phenolic content, and 63.53 and 19.87% DPPH activity, respectively. The total flavonoids content (7.23 and 14.11 mg/g) and tannin content (0.57 and 0.97 mg/g) were found in the extract of *A. tricolor* and *A. viridis*. Sarkar and Oba (2020) was observed the variation in total phenolic content 72.69 to 153.08 µg/g FW basis, total flavonoids content 30.89 to 50.95 µg/g FW basis in red amaranth. Iqbal et al. (2012) reported that 10.3 µg/g of phenolic and 27.8 µg/g of flavonoid extracted from *A. viridis* which corresponds to 14.25 DPPH, (µg/mL). Amaranth is a rich source of antioxidants in vegetables, ranging from 2.95 - 3.75 GAE, mg/100 g (Pasko et al. 2009).

Bang et al. (2021) scrutinized the variation in total polyphenol contents, total flavonoid contents, and antioxidant activities among 120 accessions of nine *Amaranthus* species. The antioxidant activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) of the 120 amaranth accessions ranged from 1.1 (*A. tricolor*) to 75.2 (*A. tricolor* mg ascorbic acid equivalents (AAE)/g in 2018, and 8.5 (*A. tricolor*) to 68.8 (*A. dubius*) mg AAE/g in 2019. Total flavonoid content (TFC) of 2018 and 2019 ranged from 21.7 (*A. caudatus*) to 52.7 (*A. hybridus*) and from 22.3 (*A.*

*viridis*) to 54.7 (*A. tricolor*), respectively (Bang et al. 2021).

### Identification of compounds in extract of *A. tricolor* and *A. viridis* by using LC-ESI-Q-TOF-MS/MS

The compounds present in the extract of *A. tricolor* and *A. viridis* are presented in Table 4. Aspartic acid, 3,4,5-Trihydroxystilbene, and caffeic acid were found in *A. tricolor*, whereas *A. viridis* shows the presence of aspartic acid n-acetyl-d-galactosamine and caffeic acid. 3,4,5-Trihydroxystilbene, known as a phytoalexin, showed its presence in grape skin and was generated by many plants. Schröter et al. (2018) found novel hydroxycinnamic acid derivatives in secondary metabolite profile of Amaranth, leaves, which confirmed by Random Amplification of Polymorphic DNA (RAPD)-PCR fingerprinting. *A. tricolor* was found very high hydroxycinnamic acid concentrations (>20 mg·g<sup>-1</sup> DW) (Schröter et al., 2018). According to Perrone et al. (2017), 3,4,5-Trihydroxystilbene possesses antiviral, antimicrobial, anticancer, anti-inflammatory, immunomodulatory, cardioprotective and neuroprotective properties.

Caffeic acid falls in the class of polyphenol derivatives, and an intermediate in the biosynthesis of lignin represents an antioxidant. Different fruit and vegetable species are a rich source of these compounds (Jakobek et al. 2007).

**Table 4:** Identification of compounds in *A. tricolor* and *A. viridis* by using LC-MS/MS profile

Name of the compounds	Retention Time (RT)	Plant metabolites	Molecular formula
<i>A. tricolor</i>			
Aspartic Acid	0.975	Amino acid	C <sub>4</sub> H <sub>7</sub> N O <sub>4</sub>
3,4,5-Trihydroxystilbene	2.083	Resveratrol (a type of natural phenol)	C <sub>14</sub> H <sub>12</sub> O <sub>3</sub>
Caffeic acid	8.865	Hydroxycinnamic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>
<i>A. viridis</i>			
Aspartic Acid	0.949	Amino acid	C <sub>4</sub> H <sub>7</sub> N O <sub>4</sub>
n-acetyl-d-galactosamine	1.62	Amino sugar derivative of galactose	C <sub>8</sub> H <sub>15</sub> N O <sub>6</sub>
Caffeic acid	28.868	Hydroxycinnamic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>



## Conclusions

In conclusion, *A. tricolor* and *A. viridis* were good sources of nutritious and non-nutritive characteristics. In extraction, water was a suitable solvent for extracting maximum polyphenolic extract from *A. tricolor* and *A. viridis*. The extraction parameter like a solvent, solvent to solid ratio, temperature, and time was useful in extracting phenolic content in *A. tricolor* 10.47mg/g and *A. viridis* 13.13mg/g by using one factor. The BBD design obtained 11.87mg/g at optimal condition solid to water ratio (1:15), temperature (30°C), and time (15 min) for *A. tricolor* and in case of *A. viridis*, it extracts 18.36mg/g at optimal condition solid to liquid ratio (1:30), temperature (30°C) and time (60 min). *A. tricolor* shows the highest antioxidant activity against DPPH compared to *A. viridis*. Caffeic acid is an active polyphenolic derivative found in both *A. tricolor* L. and *A. viridis* by using LC–ESI–Q–TOF–MS technique. The extracts thus obtained shall find a wide range of application in food fortification practices including sherbets, fruit leathers, fruit bars.

## Acknowledgments

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